

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Hypoxia and Pulmonary Hypertension

Nicoletta Charolidi and Veronica A. Carroll

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/67151>

Abstract

Vasoconstriction in response to low oxygen tension (hypoxia) in pulmonary arteries is an important physiological adaptation to reroute blood flow to areas of higher oxygenation for effective gaseous exchange. However, chronic hypoxia is a common feature of lung disease, such as chronic obstructive pulmonary disease (COPD). Hypoxic stress triggers cellular phenotypic alterations including increased proliferation and migration of vascular smooth muscle cells (VSMCs), as well as synthesis of extracellular matrix (ECM) proteins that remodel lung vasculature. Remodelling of vessels increases the risk of pulmonary hypertension (PH)—elevated pulmonary arterial pressure—and eventually right heart failure. This chapter will summarise the major pathways and mechanisms involved in hypoxia-driven pulmonary hypertension (PH).

Keywords: hypoxia, pulmonary hypertension, HIF-1 α , HIF-2 α , mTOR, VHL

1. Introduction

The main function of the cardiovascular system is to circulate and deliver oxygen to metabolically active tissues of the body. At physiologically normal oxygen levels, the pulmonary vasculature of healthy individuals is highly distensible, allowing the cardiac output to adjust to levels of activity. In varying degrees of oxygen availability, as in different altitudes, adaptive cardiovascular responses are employed. In acute hypoxia (short, transient reduction in oxygen tension), the pulmonary vascular bed constricts rapidly [1]. When oxygen levels are restored, it dilates again in a swift and reversible manner. With a sustained hypoxic exposure (hours to days), the response is different. There is a loss of pulmonary distensibility, increased arterial pressure, tachycardia and increased workload for the right cardiac ventricle. In return to normoxic conditions, there is, at least in the short term, a limited reversibility of these effects. The Operation Everest II study [2] demonstrated this phenomenon by monitoring the pulmonary vascular pressure of healthy individuals who were exposed to progressive

partially pressured oxygen over a period of a few weeks. However, for high-altitude populations, such as the Tibetans, this is not the case. Due to natural selection and adaptation over many thousands of years living under low oxygen conditions, Tibetans have altered oxygen-sensing mechanisms and pulmonary vascular resistance to sustained hypoxia (discussed later in this chapter) [3].

Healthy, native sea-level dwellers, who move to high altitude, develop high pulmonary arterial pressure, but with time, in the majority of cases, it stabilises and becomes well tolerated [4]. By contrast, people with pre-existing lung pathologies, such as chronic obstructive pulmonary disease (COPD), cystic fibrosis, idiopathic pulmonary fibrosis, bronchiectasis or restrictive chest wall abnormalities, are at risk of developing pulmonary hypertension (PH). Chronic PH lowers quality of life and decreases life expectancy for the affected individuals [5–8].

The pathophysiology of hypoxia-associated PH is characterised by extensive vascular remodelling that leads to arterial narrowing rather than reversible vessel vasoconstriction (**Figure 1**). Processes that take place include endothelial cell dysfunction, muscularisation of normally non-muscular arteries, phenotypic switching and proliferation of vascular smooth muscle cells (VSMCs), increased extracellular matrix deposition and erythrocytosis [7, 9, 10]. In this chapter, recent developments in mechanistic aspects underlying hypoxia-induced pathophysiological changes in PH will be briefly summarised.

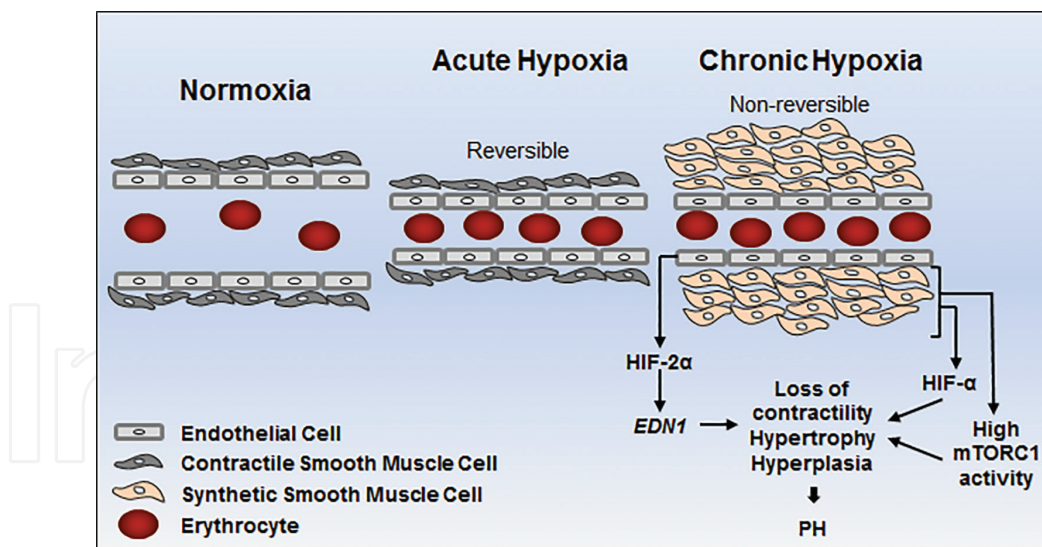


Figure 1. Schematic representation of pulmonary arterial responses to normoxia, acute hypoxia and chronic hypoxia. With acute and chronic hypoxia, the pulmonary artery undergoes vasoconstriction. In the case of acute hypoxia, the artery can reversibly dilate. But in chronic hypoxia, the artery undergoes nonreversible vascular remodelling characterised by intimal thickening due to VSMC dedifferentiation (loss of contractility, hypertrophy and hyperplasia). Additionally, there is distal muscularisation of non-muscular vessels, a settled-in endothelial cell dysfunction and erythrocytosis. Activation of HIF-1 α and HIF-2 α as well as over-activation of mTORC1 contributes to VSMC dedifferentiation and the establishment of hypoxic PH. Abbreviations: HIF-1 α , hypoxia-inducible factor 1 α ; HIF-2 α , hypoxia-inducible factor 2 α ; mTORC1, mechanistic target of rapamycin complex 1; PH, pulmonary hypertension.

2. Role of endothelial cell dysfunction

Endothelial cells in pulmonary vessels first sense hypoxic stress. Having a role in maintaining homeostasis, endothelial cells contribute to reducing the vascular tone in order for vasoconstriction to take place and regulate vessel adaptation to increased blood flow [11]. In healthy individuals, the endothelium is responsible for the balanced expression of vasoactive mediators that have either vasodilator ability, such as nitric oxide (NO) and prostacyclin (PGI₂), or vasoconstrictive properties, such as endothelin-1 (ET-1) [11–14]. ET-1 is released abluminally and triggers vasoconstriction through binding to its VSMC receptors ET_A and ET_B [15]. However, when ET-1 binds to its endothelial ET_B receptor, it can induce vasodilation through NO and PGI₂ recruitment [15], while this route also serves for ET-1 clearance from the lung [16].

In pathological PH, as in COPD, endothelial cell dysfunction is one of the major contributing factors for the progression of the condition. It has been found that endothelial NO synthase (eNOS), the enzyme responsible for NO production, as well as prostacyclin synthase, the enzyme responsible for PGI₂ production, is markedly diminished in patients with COPD [12, 17]. Furthermore, ET-1 has been reported to have an increased expression in the lungs of patients with PH and is a therapeutic target [14]. ET-1, as well as being a potent vasoconstrictor, is also a VSMC mitogen, acting through smooth muscle ET_A and ET_B receptors [15]. So in effect, during hypoxic endothelial dysregulation, the pathogenic excess of ET-1 maintains vessel constriction and VSMC proliferation.

3. Phenotypic switching of vascular smooth muscle cells

In hypoxia, the highly plastic VSMCs switch from a contractile to a synthetic phenotype, which is characterised by increased proliferation and extracellular matrix deposition [18]. Differentiated smooth muscle cells express a repertoire of contractile proteins, signalling molecules and receptors for their primary function of vessel contraction. These contractile VSMCs have little capacity for proliferation, protein synthesis or migration [18]. However, pulmonary VSMCs, under chronic hypoxic stimulation, switch to a synthetic state exhibiting hypertrophy, hyperplasia, loss of contractility and migration, contributing to the enlargement of the arterial intimal layer (**Figure 1**) and in the muscularisation of non-muscular pulmonary vessels [9]. Additionally, there is a deposition of collagen and elastic fibres. In extreme cases, the excessive VSMC proliferation can progress from vascular lesions to calcification. These phenomena seem to correlate with the degree of PH extent and COPD severity [19–21].

The endothelial dysfunction that takes place in PH may also contribute to the dedifferentiation and proliferation of VSMCs [22]. Specifically, dysregulated endothelial cells can cause alterations in AKT signalling in VSMCs, which in turn triggers their phenotypic switch [23]. This pathway is also affected by aberrant regulation of the mechanistic target of rapamycin (mTOR) pathway (discussed later in this chapter).

4. Hypoxia and pulmonary hypertension

The major cellular oxygen-sensing mechanism implicated in hypoxia-induced pulmonary hypertension is the hypoxia-inducible factor (HIF) pathway. HIFs are transcription factors that induce the activation of some several hundred genes in response to hypoxia [24]. Initially identified as regulators of erythropoietin (EPO), the hormone responsible for increased red blood cells in response to low oxygen levels, HIFs have since been found to regulate expression of genes that are important for angiogenesis, cellular metabolism, cardiovascular development and cardiovascular control [24–26].

In low oxygen conditions, HIFs bind DNA as heterodimeric complexes of alpha (HIF- α) and beta (HIF- β) subunits, with HIF- α being the subunit regulated by oxygen tension [27]. Higher animals have a series of isoforms for each of the HIF subunits as a result of gene evolutionary duplications [24]. In humans, there are three paralogs of HIF- α —HIF-1 α , HIF-2 α and HIF-3 α —with the first two members being the best characterised [24, 25]. The expression of HIF-1 α and HIF-2 α is differentially regulated, while their balance is believed to be important for tissue-specific differences in oxygen sensing [25]. They both bind to the same DNA consensus (RCGTG) in hypoxia-response elements of the genome, but they only induce partially overlapping sets of genes [27, 28].

In normoxic conditions, the HIF- α subunit is hydroxylated by Fe(II) prolyl hydroxylase domain (PHD) enzymes (PHD1, PHD2 and PHD3 or otherwise known as EglN2, EglN1 and EglN3) that use 2-oxoglutarate and Fe²⁺ as substrates [29]. After hydroxylation by PHDs, HIF- α is recognised and bound by the von Hippel-Lindau (VHL) protein, a ubiquitin E3 ligase, which marks HIF- α for proteasomal degradation. In hypoxia, PHD enzymes are inactive allowing HIF- α subunits to translocate to the nucleus and activate HIF target genes. HIFs are further regulated by factor-inhibiting HIF (FIH)-mediated asparaginyl hydroxylation, which impairs their recruitment to transcriptional complexes [30].

Mouse models of HIF-1 α and HIF-2 α have illustrated that the HIF pathway is critically important for the pulmonary hypoxic response and the development of PH. Heterozygous deficiency of either HIF-1 α or HIF-2 α allele in mice does not affect their life span, and these animals are largely normal in unstressed, normal oxygen conditions. In response to chronic hypoxia (10% for 3 weeks), HIF-1 α ^{+/-} mice exhibit an attenuated PH with a low rise in right ventricular pressure and right ventricular hypertrophy [31]. Interestingly, heterozygous HIF-2 α ^{+/-} mice, exposed to 10% oxygen for 10 weeks, showed a complete lack of any PH manifestation [32]. Of note, animals with hetero- or homozygous mutations in stabilising HIF-2 α spontaneously developed progressive PH [33]. These studies all indicate a pathological role of both HIF- α subunits in PH development.

Cell-type-specific inactivation of HIF- α with the use of a variety of promoters has also been studied but with some variable results, which may be due to the method of HIF- α manipulations and/or the use of different mouse strains [34–36]. Nevertheless, there seems to be a clear link between HIFs and PH, since studies from human genetics, including several populations that have adapted to different altitudes, have demonstrated the importance of HIF-2 α in pulmonary response to hypoxia and PH pathophysiology [37].

The Tibetans, who have lived for at least 25,000 years in 4000 m elevation and continuously inspired partially pressured oxygen (~80 mmHg), have been identified to have a number of single-nucleotide polymorphisms in close-to-one-another loci near the gene *EPAS1*, which encodes HIF-2 α [38]. HIF-2 α is the subunit responsible for EPO regulation and in turn erythropoiesis. Tibetans manifest blunted PH and reduced erythropoiesis at high altitude. At sea level, they manifest a lower pulmonary arterial pressure in response to hypoxia when compared with other populations [39, 40]. Recently, a missense mutation in *PHD2* (*EGLN1*) was identified which allows for increased PHD2 activity under hypoxic conditions, thereby decreasing HIF- α stabilisation and reducing erythropoiesis at altitude [41].

Further evidence for a role for HIF-2 α in PH comes from another human genetic study, which showed that an activating HIF-2 α mutation (G→A substitution in position 2097) caused erythrocytosis with elevated total red cell volume and PH in an affected family [42].

5. VHL and pulmonary hypertension

The VHL protein is a tumour suppressor and an essential component for the clearance of HIF- α through the ubiquitin-proteasomal degradation pathway [24, 43]. A number of VHL mutations have been described that result in aberrant induction of HIF target genes, due to the loss of function of VHL and in turn to the loss of HIF- α regulation. VHL mutations are associated with VHL syndrome, which is a hereditary condition, characterised by highly vascularised tumours within specific tissues, including the renal, retinal and central nervous system [44]. However, a small number of VHL mutations (R200W, D126N, S183L, D126N) are associated with development of Chuvash polycythemia (CP) [45–47]. CP is a rare autosomal recessive condition that is endemic to the population in Chuvashia, Russia and in the island of Ischia, Italy [46, 48]. Chuvash patients manifest increased haemoglobin and haematocrit with elevated levels of EPO, as well as increased expression of vascular endothelial growth factor (VEGF) and ET-1, which are HIF- α target genes [45–49]. In addition, these patients are highly susceptible to both arterial and venous thrombosis and can develop mild to severe PH [45–49].

The importance of HIF-2 α isoform in the regulation of pulmonary vascular control has also been demonstrated by the use of a mouse model of CP [50]. This model carries a hypomorphic VHL allele (with an R200W substitution) and recapitulates all symptoms of the human CP phenotype. Interestingly, when these mice are crossed with HIF-2 $\alpha^{+/-}$ or HIF-1 $\alpha^{+/-}$ strains for heterozygous deficiency in either of the two HIF- α , they manifest an ameliorated PH phenotype for suppressed HIF-2 α , but not for HIF-1 α .

Comparison of CP and HIF-2 α gain-of-function mutation human phenotypes has additionally shown that the latter condition somehow manifests more moderate symptoms than the first. The explanation for this may be that, in CP, both HIF- α subunits are upregulated, and therefore, there may be an additive effect [51]. Furthermore, VHL has a number of HIF- α -independent functions that may also play a role in the CP phenotype.

6. New advances: hypoxic induction of zinc transporters

Zinc, an essential dietary element, plays an important cytoprotective role for the lung by sheltering the pulmonary epithelium from extrinsic activation of apoptotic pathways following acute lung injury [52]. Zinc transporters are responsible for zinc cellular uptake and homeostasis [53]. A recent linkage analysis study that compared a PH-resistant rat strain, Fisher 344 (F344), with the Wistar Kyoto (WKY) strain identified the gene *Slc39a12*, which encodes the ZIP12 zinc transporter, as a major regulator of hypoxia-induced pulmonary vascular remodelling [53]. In the F344 strain, this gene lacks a crucial thymidine, which leads to a frameshift mutation in exon 11 and renders translation of the protein redundant. ZIP12 is normally expressed in endothelial, interstitial and VSMCs, but its expression increases in remodelled pulmonary vessels following hypoxia-induced PH [53]. ZIP12 is likely a HIF target gene since both HIF-1 α and HIF-2 α were detected bound to ZIP12 hypoxia-response element. The investigators of this study further generated a ZIP12^{-/-} rat model for comparison with the original F344 and WKY strains and found that genetic disruption of ZIP12 recapitulates the phenotype of the PH-resistant F344 strain under conditions of hypoxia.

Zinc-binding motifs have been considered as potential PH drug-therapeutic targets with phosphodiesterase type 5 (PDE5) and histone deacetylases as examples [54, 55]. Zinc is a structural component of a number of intracellular enzymes, transcription factors, other proteins and cofactors and is a putative drug target for PH.

7. Role of hypoxia-inducible microRNAs in pulmonary hypertension

MicroRNAs (miRNAs) are small non-coding RNA molecules (about 21 nucleotides long) that regulate gene expression post-transcriptionally. Hypoxic stimulation of a variety of human cell types has shown induction of more than 90 miRNAs [56], with altered expression of some of these miRNAs involved in VSMC remodelling and endothelial cell dysfunction in PH [57].

MiRNAs that have been causally implicated in PH include miR-204, miR-138, miR-21 and miR-130/miR-301, among others (**Table 1**). MiR-204 has been shown to be downregulated in VSMCs of patients suffering from PH, as well as in mouse models of the disease [58, 59]. The degree of miR-204 suppression has been found to be inversely proportional to the degree of pulmonary artery resistance and pressure, while compensating for the loss of miR-204 through nebulisation in PH patients has been shown to reverse the VSMC proliferative and anti-apoptotic phenotype [59]. MiR-204 is involved in the activation of the nuclear factor of activated T cell (NFAT) pathway, the Rho pathway, VSMC proliferation and resistance to apoptosis, as well as downregulation of transcripts such as bone morphogenetic protein receptor type II (BMPRII) and interleukin-6 (IL-6) [60–62]. Also, miR-204 regulates the expression of the Runt-related transcription factor 2 (RUNX2), which has been shown to stabilise HIF-1 α in chondrocytes by competing with VHL [20, 63]. In the context of hypoxia, RUNX2 is upregulated, since miR-204 is downregulated, and therefore sustains HIF-1 α activation,

MicroRNA	Change in PH	Target transcripts	Cellular function, process or pathway affected	Ref.
miR-204	↓	BMPR2, IL-6, RUNX2 among others	Activation of NFAT pathway, VSMC proliferation, resistance to apoptosis, Rho pathway, HIF-1 α pathway	[20, 58–63]
miR-138	↑	HIF-1 α , S100A1	HIF-1 α pathway, endothelial regulation of vasomotor tone	[64]
miR-21	↑	PDCD4, SPRY2, PPAR α	VSMC proliferation, resistance to apoptosis	[61, 65–67]
miR-130/301	↑	PPAR γ which leads to subordinate gene targets and other miRNAs	Master regulator of cell proliferation and apoptosis in PH ↓ miR-204	[68]

Table 1. MicroRNAs that are causally implicated in PH.

which in turn contributes to aberrant VSMC proliferation, resistance to apoptosis and their transdifferentiation to osteoblast-like cells [20].

MiR-138 is upregulated by hypoxia and suppresses HIF-1 α [64]. However, its upregulation also contributes to endothelial cell dysfunction in PH by downregulating the small EF-hand Ca²⁺-binding protein S100A1 that relays Ca²⁺ oscillations, controlling vascular tone responses [64].

MiR-21 expression has been found to be upregulated in both pulmonary VSMC and endothelial cells during hypoxic conditions [61, 65]. This upregulation, in turn, leads to downregulation of programmed cell death protein 4 (PDCD4), sprouty homolog 2 (SPRY2) and peroxisome proliferator-activated receptor- α (PPAR α), which when dysregulated play a role in the increased proliferation and resistance to apoptosis [65–67]. Treatment of mice with anti-miR-21 during hypoxia showed an improvement in distal pulmonary artery muscularisation [69]. However, miR-21 has also been shown to have a protective effect during PH [61]. Using VHL-null mice, IL-6 transgenic mice, pulmonary vessels from patients with PH as well as deficient (miR-21^{-/-}) or miR-21 overexpression (miR-21^{+/+}) mouse models, it has been demonstrated that miR-21 loss of function causes onset of PH [61]. Specifically, miR-21 deletion showed exaggerated pulmonary vascular remodelling, whereas in mice overexpressing miR-21, these disease-associated phenotypes were abolished [61].

The family of miR-130/301 is also upregulated in pulmonary VSMCs and the endothelium in hypoxia, as well as in the lungs of mice with PH due to chronic hypoxic exposure [68]. This upregulation is mediated by HIF-2 α and Oct-4. MiR-130/301 is a master regulator miRNA subordinating other miRNA pathways, and, for instance, it suppresses miR-204 [68].

miR-223, miR-17, miR-130, miR-145, miR-424 and miR503 are also involved in the pathophysiology of PH (reviewed in Ref. [70]). So far, PH animal models have helped greatly in these studies, but the exact role and balance for each of these miRNAs in human PH have not been fully elucidated.

8. mTOR signalling in hypoxia-induced pulmonary hypertension

Mechanistic target of rapamycin (mTOR) is a cellular hub that controls growth factor signalling and nutrient sensing to regulate cell growth, proliferation, metabolism and survival [71]. mTOR is a protein kinase that is the catalytic component of two functionally distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [72, 73]. mTORC1 is composed of mTOR, Raptor, LST8/G β L, PRAS40 and DEP domain containing mTOR-interacting protein (DEPTOR), and its activity is stimulated by growth factor signals to regulate protein synthesis through 4E-BP1/BP2 and the S6 kinases, S6K1 and S6K2 [74, 75]. By contrast, mTORC2, which comprises mTOR, Rictor, LST8/G β L, DEPTOR, SIN1 and PRR5, regulates cytoskeletal organisation [76, 77] and has a role in phosphorylation of protein kinase C (PKC), protein kinase B (PKB) and serum- and glucocorticoid-induced protein kinase (SGK) to promote cell survival and cell cycle progression [78–80].

Aberrant mTOR activity has a well-characterised role in promoting proliferative diseases including cancer and smooth muscle cell pathologies [71]. mTORC1 signalling is activated following vascular injury promoting Vihibitor, rapamycin, promotes smooth muscle cell (SMC) remodelling. Accordingly, mTOR inhibitors are widely used in drug-eluting stents to prevent restenosis. In addition, mTOR also regulates the differentiation state of VSMCs since the mTOR inhibitor, rapamycin, promotes SMC differentiation and expression of contractile proteins [81]. mTORC1 activity is low in differentiated contractile VSMCs but becomes activated by growth factors and is thought to contribute to the change towards a synthetic phenotype that is characterised by increased SMC proliferation and migration. As such, rapamycin analogues may have therapeutic potential for treating PH.

The relationship between hypoxic conditions and mTOR is complex and depends, in part, on cellular context. Many cell types respond to prolonged periods of hypoxia by inactivating energy-intensive processes such as protein synthesis and proliferation, and accordingly mTOR is downregulated [82]. By contrast, the vasculature responds to long-term hypoxia by promoting new blood vessel growth—angiogenesis, which in turn, restores O₂ to deprived tissues. Hypoxic stress is a key driving force in the vascular remodelling observed in pulmonary hypertension, and HIFs activate pulmonary artery endothelial and smooth muscle cell proliferation, which is mediated by both mTORC1 and mTORC2 [83–85]. Currently, the mechanisms by which hypoxia/HIFs signal to activate mTOR in ECs and VSMCs are poorly understood [86–90].

9. Conclusion

Severe PH associated with hypoxic lung disease is a life-threatening condition with poor survival rates. Despite significant advances in targeted therapeutics for PH, randomised clinical trial data for this particular group of patients are scarce, and it is not clear whether endothelin receptor antagonists will benefit patients with hypoxia-associated PH. Importantly, recent genetic studies identifying mutations in the oxygen-sensing machinery have provided new mechanistic insights into the aetiology of PH. Further studies are required to determine whether specific targeting of HIF-2 α will provide additional therapeutic benefit for this complex disease.

Author details

Nicoletta Charolidi and Veronica A. Carroll*

*Address all correspondence to: vc Carroll@sgul.ac.uk

Vascular Biology Research Centre, Molecular and Clinical Sciences Research Institute, St George's, University of London, London, UK

References

- [1] Euler U, Liljestrand G. Observations on the pulmonary arterial blood pressure in the cat. *Acta Physiol Scand.* 1946;12:301–20.
- [2] Groves BM, Reeves JT, Sutton JR, Wagner PD, Cymerman A, Malconian MK, et al. Operation Everest II: elevated high-altitude pulmonary resistance unresponsive to oxygen. *J Appl Physiol* (1985). 1987 Aug;63(2):521–30.
- [3] Aldenderfer M. Peopling the Tibetan plateau: insights from archaeology. *High Alt Med Biol.* 2011;12(2):141–7.
- [4] West JB, American College of Physicians, American Physiological Society. The physiologic basis of high-altitude diseases. *Ann Intern Med.* 2004 Nov 16;141(10):789–800.
- [5] Seeger W, Adir Y, Barberà JA, Champion H, Coghlan JG, Cottin V, et al. Pulmonary hypertension in chronic lung diseases. *J Am Coll Cardiol.* 2013 Dec 24;62 (25 Suppl):D109–16.
- [6] Wells JM, Washko GR, Han MK, Abbas N, Nath H, Mamary AJ, et al. Pulmonary arterial enlargement and acute exacerbations of COPD. *N Engl J Med.* 2012 Sep 6;367(10):913–21.
- [7] Blanco I, Piccari L, Barberà JA. Pulmonary vasculature in COPD: the silent component. *Respirology.* 2016 Aug;21(6):984–94.
- [8] Wells JM, Farris RF, Gosdin TA, Dransfield MT, Wood ME, Bell SC, et al. Pulmonary artery enlargement and cystic fibrosis pulmonary exacerbations: a cohort study. *Lancet Respir Med.* 2016 Aug;4(8):636–45.
- [9] Stenmark KR, Fagan KA, Frid MG. Hypoxia-induced pulmonary vascular remodeling: cellular and molecular mechanisms. *Circ Res.* 2006 Sep 29;99(7):675–91.
- [10] Kylhammar D, Rådegran G. The principal pathways involved in the in vivo modulation of hypoxic pulmonary vasoconstriction, pulmonary arterial remodelling and pulmonary hypertension. *Acta Physiol (Oxf).* 2016 Jul 6;Epub ahead of print.
- [11] Stamler JS, Loh E, Roddy MA, Currie KE, Creager MA. Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. *Circulation.* 1994 May;89(5):2035–40.
- [12] Tudor RM, Cool CD, Geraci MW, Wang J, Abman SH, Wright L, et al. Prostacyclin synthase expression is decreased in lungs from patients with severe pulmonary hypertension. *Am J Respir Crit Care Med.* 1999 Jun;159(6):1925–32.

- [13] Barberà JA, Roger N, Roca J, Rovira I, Higenbottam TW, Rodriguez-Roisin R. Worsening of pulmonary gas exchange with nitric oxide inhalation in chronic obstructive pulmonary disease. *Lancet* (London, England). 1996 Feb 17;347(8999):436–40.
- [14] Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med*. 1993 Jun 17;328(24):1732–9.
- [15] Bialecki RA, Fisher CS, Murdoch WW, Barthlow HG, Bertelsen DL. Functional comparison of endothelin receptors in human and rat pulmonary artery smooth muscle. *Am J Physiol*. 1997 Feb;272(2 Pt 1):L211–8.
- [16] Fukuroda T, Fujikawa T, Ozaki S, Ishikawa K, Yano M, Nishikibe M. Clearance of circulating endothelin-1 by ETB receptors in rats. *Biochem Biophys Res Commun*. 1994 Mar 30;199(3):1461–5.
- [17] Yang Q, Shigemura N, Underwood MJ, Hsin M, Xue H-M, Huang Y, et al. NO and EDHF pathways in pulmonary arteries and veins are impaired in COPD patients. *Vascul Pharmacol*. 2012;57(2–4):113–8.
- [18] Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev*. 2004 Jul;84(3):767–801.
- [19] Carlsen J, Hasseriis Andersen K, Boesgaard S, Iversen M, Steinbrüchel D, Bøgelund Andersen C. Pulmonary arterial lesions in explanted lungs after transplantation correlate with severity of pulmonary hypertension in chronic obstructive pulmonary disease. *J Heart Lung Transplant*. 2013 Mar;32(3):347–54.
- [20] Ruffenach G, Chabot S, Tanguay VF, Courboulain A, Boucherat O, Potus F, et al. Role for Runt-related transcription factor 2 in proliferative and calcified vascular lesions in pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2016 Nov 15;194(10):1273–85.
- [21] Santos S, Peinado VI, Ramírez J, Melgosa T, Roca J, Rodriguez-Roisin R, et al. Characterization of pulmonary vascular remodelling in and patients with mild COPD. *Eur Respir J*. 2002 Apr;19(4):632–8.
- [22] Powell RJ, Cronenwett JL, Fillinger MF, Wagner RJ, Sampson LN. Endothelial cell modulation of smooth muscle cell morphology and organizational growth pattern. *Ann Vasc Surg*. 1996 Jan;10(1):4–10.
- [23] Brown DJ, Rzucidlo EM, Merenick BL, Wagner RJ, Martin KA, Powell RJ. Endothelial cell activation of the smooth muscle cell phosphoinositide 3-kinase/Akt pathway promotes differentiation. *J Vasc Surg*. 2005 Mar;41(3):509–16.
- [24] Bishop T, Ratcliffe PJ. HIF hydroxylase pathways in cardiovascular physiology and medicine. *Circ Res*. 2015 Jun 19;117(1):65–79.
- [25] Semenza GL. Oxygen sensing, homeostasis, and disease. *N Engl J Med*. 2011 Aug 11;365(6):537–47.

- [26] Maxwell PH, Pugh CW, Ratcliffe PJ. Inducible operation of the erythropoietin 3' enhancer in multiple cell lines: evidence for a widespread oxygen-sensing mechanism. *Proc Natl Acad Sci U S A*. 1993 Mar 15;90(6):2423–7.
- [27] Hu CJ, Wang LY, Chodosh LA, Keith B, Simon MC. Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. *Mol Cell Biol*. 2003 Dec;23(24):9361–74.
- [28] Schödel J, Oikonomopoulos S, Ragoussis J, Pugh CW, Ratcliffe PJ, Mole DR. High-resolution genome-wide mapping of HIF-binding sites by ChIP-seq. *Blood*. 2011 Jun 9;117(23):e207–17.
- [29] Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science*. 2001 Nov 9;294(5545):1337–40.
- [30] Mahon PC, Hirota K, Semenza GL. FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev*. 2001 Oct 15;15(20):2675–86.
- [31] Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, et al. Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha. *J Clin Invest*. 1999 Mar;103(5):691–6.
- [32] Brusselmans K, Compennolle V, Tjwa M, Wiesener MS, Maxwell PH, Collen D, et al. Heterozygous deficiency of hypoxia-inducible factor-2alpha protects mice against pulmonary hypertension and right ventricular dysfunction during prolonged hypoxia. *J Clin Invest*. 2003 May;111(10):1519–27.
- [33] Tan Q, Kerestes H, Percy MJ, Pietrofesa R, Chen L, Khurana TS, et al. Erythrocytosis and pulmonary hypertension in a mouse model of human HIF2A gain of function mutation. *J Biol Chem*. 2013 Jun 14;288(24):17134–44.
- [34] Ball MK, Waypa GB, Mungai PT, Nielsen JM, Czech L, Dudley VJ, et al. Regulation of hypoxia-induced pulmonary hypertension by vascular smooth muscle hypoxia-inducible factor-1 α . *Am J Respir Crit Care Med*. 2014 Feb 1;189(3):314–24.
- [35] Kim Y-M, Barnes EA, Alvira CM, Ying L, Reddy S, Cornfield DN. Hypoxia-inducible factor-1 α in pulmonary artery smooth muscle cells lowers vascular tone by decreasing myosin light chain phosphorylation. *Circ Res*. 2013 Apr 26;112(9):1230–3.
- [36] Skuli N, Liu L, Runge A, Wang T, Yuan L, Patel S, et al. Endothelial deletion of hypoxia-inducible factor-2alpha (HIF-2alpha) alters vascular function and tumor angiogenesis. *Blood*. 2009 Jul 9;114(2):469–77.
- [37] Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell*. 2012 Feb 3;148(3):399–408.
- [38] Beall CM, Cavalleri GL, Deng L, Elston RC, Gao Y, Knight J, et al. Natural selection on EPAS1 (HIF2alpha) associated with low hemoglobin concentration in Tibetan highlanders. *Proc Natl Acad Sci U S A*. 2010 Jun 22;107(25):11459–64.

- [39] Petousi N, Croft QP, Cavalleri GL, Cheng HY, Formenti F, Ishida K, et al. Tibetans living at sea level have a hyporesponsive hypoxia-inducible factor system and blunted physiological responses to hypoxia. *J Appl Physiol*. 2014 Apr 1;116(7):893–904.
- [40] Simonson TS, Yang Y, Huff CD, Yun H, Qin G, Witherspoon DJ, et al. Genetic evidence for high-altitude adaptation in Tibet. *Science*. 2010 Jul 2;329(5987):72–5.
- [41] Lorenzo FR, Huff C, Myllymäki M, Olenchok B, Swierczek S, Tashi T, et al. A genetic mechanism for Tibetan high-altitude adaptation. *Nat Genet*. 2014;46(9):951–6.
- [42] Gale DP, Harten SK, Reid CDL, Tuddenham EG, Maxwell PH. Autosomal dominant erythrocytosis and pulmonary arterial hypertension associated with an activating HIF2 alpha mutation. *Blood*. 2008 Aug 1;112(3):919–21.
- [43] Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*. 1999 May 20;399(6733):271–5.
- [44] Chittiboina P, Lonser RR. Von Hippel-Lindau disease. *Handb Clin Neurol*. 2015;132:139–56.
- [45] Sarangi S, Lanikova L, Kapralova K, Acharya S, Swierczek S, Lipton JM, et al. The homozygous VHL(D126N) missense mutation is associated with dramatically elevated erythropoietin levels, consequent polycythemia, and early onset severe pulmonary hypertension. *Pediatr Blood Cancer*. 2014 Nov;61(11):2104–6.
- [46] Ang SO, Chen H, Hirota K, Gordeuk VR, Jelinek J, Guan Y, et al. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. *Nat Genet*. 2002 Dec;32(4):614–21.
- [47] Bond J, Gale DP, Connor T, Adams S, de Boer J, Gascoyne DM, et al. Dysregulation of the HIF pathway due to VHL mutation causing severe erythrocytosis and pulmonary arterial hypertension. *Blood*. 2011 Mar 31;117(13):3699–701.
- [48] Perrotta S, Nobili B, Ferraro M, Migliaccio C, Borriello A, Cucciolla V, et al. Von Hippel-Lindau-dependent polycythemia is endemic on the island of Ischia: identification of a novel cluster. *Blood*. 2006 Jan 15;107(2):514–9.
- [49] Smith TG, Brooks JT, Balanos GM, Lappin TR, Layton DM, Leedham DL, et al. Mutation of von Hippel-Lindau tumour suppressor and human cardiopulmonary physiology. *PLoS Med*. 2006 Jul;3(7):e290.
- [50] Hickey MM, Richardson T, Wang T, Mosqueira M, Arguiri E, Yu H, et al. The von Hippel-Lindau Chuvash mutation promotes pulmonary hypertension and fibrosis in mice. *J Clin Invest*. 2010 Mar;120(3):827–39.
- [51] Formenti F, Beer PA, Croft QP, Dorrington KL, Gale DP, Lappin TRJ, et al. Cardiopulmonary function in two human disorders of the hypoxia-inducible factor (HIF) pathway: von Hippel-Lindau disease and HIF-2alpha gain-of-function mutation. *FASEB J*. 2011 Jun;25(6):2001–11.

- [52] Zalewski PD, Forbes IJ, Betts WH. Correlation of apoptosis with change in intracellular labile Zn(II) using zinquin [(2-methyl-8-p-toluenesulphonamido-6-quinolyloxy)acetic acid], a new specific fluorescent probe for Zn(II). *Biochem J*. 1993 Dec 1;296(Pt 2):403-8.
- [53] Zhao L, Oliver E, Maratou K, Atanur SS, Dubois OD, Cotroneo E, et al. The zinc transporter ZIP12 regulates the pulmonary vascular response to chronic hypoxia. *Nature*. 2015 Aug 20;524(7565):356–60.
- [54] Zhao L, Mason NA, Morrell NW, Kojonazarov B, Sadykov A, Maripov A, et al. Sildenafil inhibits hypoxia-induced pulmonary hypertension. *Circulation*. 2001 Jul 24;104(4):424–8.
- [55] Zhao L, Chen CN, Hajji N, Oliver E, Cotroneo E, Wharton J, et al. Histone deacetylation inhibition in pulmonary hypertension: therapeutic potential of valproic acid and suberoylanilide hydroxamic acid. *Circulation*. 2012 Jul 24;126(4):455–67.
- [56] Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res*. 2014 Jan;42(Database issue):D68–73.
- [57] White K, Loscalzo J, Chan SY. Holding our breath: the emerging and anticipated roles of microRNA in pulmonary hypertension. *Pulm Circ*. 2012 Jul;2(3):278–90.
- [58] Caruso P, MacLean MR, Khanin R, McClure J, Soon E, Southgate M, et al. Dynamic changes in lung microRNA profiles during the development of pulmonary hypertension due to chronic hypoxia and monocrotaline. *Arterioscler Thromb Vasc Biol*. 2010 Apr;30(4):716–23.
- [59] Courboulin A, Paulin R, Giguère NJ, Saksouk N, Perreault T, Meloche J, et al. Role for miR-204 in human pulmonary arterial hypertension. *J Exp Med*. 2011 Mar 14;208(3):535–48.
- [60] Bonnet S, Rochefort G, Sutendra G, Archer SL, Haromy A, Webster L, et al. The nuclear factor of activated T cells in pulmonary arterial hypertension can be therapeutically targeted. *Proc Natl Acad Sci U S A*. 2007 Jul 3;104(27):11418–23.
- [61] Parikh VN, Jin RC, Rabello S, Gulbahce N, White K, Hale A, et al. MicroRNA-21 integrates pathogenic signaling to control pulmonary hypertension: results of a network bioinformatics approach. *Circulation*. 2012 Mar 27;125(12):1520–32.
- [62] Tudor RM, Marecki JC, Richter A, Fijalkowska I, Flores S. Pathology of pulmonary hypertension. *Clin Chest Med*. 2007 Mar;28(1):23–42, vii.
- [63] Lee SH, Che X, Jeong JH, Choi JY, Lee YJ, Lee YH, et al. Runx2 protein stabilizes hypoxia-inducible factor-1 α through competition with von Hippel-Lindau protein (pVHL) and stimulates angiogenesis in growth plate hypertrophic chondrocytes. *J Biol Chem*. 2012 Apr 27;287(18):14760–71.
- [64] Sen A, Ren S, Lerchenmüller C, Sun J, Weiss N, Most P, et al. MicroRNA-138 regulates hypoxia-induced endothelial cell dysfunction by targeting S100A1. *PLoS One*. 2013 Nov 11;8(11):e78684.
- [65] Sarkar J, Gou D, Turaka P, Viktorova E, Ramchandran R, Raj JU. MicroRNA-21 plays a role in hypoxia-mediated pulmonary artery smooth muscle cell proliferation and migration. *Am J Physiol Lung Cell Mol Physiol*. 2010 Dec;299(6):L861–71.

- [66] Cheng Y, Zhu P, Yang J, Liu X, Dong S, Wang X, et al. Ischaemic preconditioning-regulated miR-21 protects heart against ischaemia/reperfusion injury via anti-apoptosis through its target PDCD4. *Cardiovasc Res*. 2010 Aug 1;87(3):431–9.
- [67] Davis BN, Hilyard AC, Lagna G, Hata A. SMAD proteins control DROSHA-mediated microRNA maturation. *Nature*. 2008 Jul 3;454(7200):56–61.
- [68] Bertero T, Lu Y, Annis S, Hale A, Bhat B, Saggar R, et al. Systems-level regulation of microRNA networks by miR-130/301 promotes pulmonary hypertension. *J Clin Invest*. 2014 Aug;124(8):3514–28.
- [69] Yang S, Banerjee S, Freitas Ad, Cui H, Xie N, Abraham E, et al. miR-21 regulates chronic hypoxia-induced pulmonary vascular remodeling. *Am J Physiol Lung Cell Mol Physiol*. 2012 Mar 15;302(6):L521–9.
- [70] Mohsenin V. The emerging role of microRNAs in hypoxia-induced pulmonary hypertension. *Sleep Breath*. 2016 Sep;20(3):1059–67.
- [71] Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell*. 2012 Apr 13;149(2):274–93.
- [72] Sciarretta S, Volpe M, Sadoshima J. Mammalian target of rapamycin signaling in cardiac physiology and disease. *Circ Res*. 2014 Jan 31;114(3):549–64.
- [73] Ratcliffe PJ. Oxygen sensing and hypoxia signalling pathways in animals: the implications of physiology for cancer. *J Physiol*. 2013 Apr 15;591(8):2027–42.
- [74] Corradetti MN, Guan KL. Upstream of the mammalian target of rapamycin: do all roads pass through mTOR?. *Oncogene*. 2006 Oct 16;25(48):6347–60.
- [75] Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell*. 2010 Oct 22;40(2):310–22.
- [76] Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol*. 2004 Jul 27;14(14):1296–302.
- [77] Liu L, Das S, Losert W, Parent CA. mTORC2 regulates neutrophil chemotaxis in a cAMP- and RhoA-dependent fashion. *Dev Cell*. 2010 Dec 14;19(6):845–57.
- [78] Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science*. 2005 Feb 18;307(5712):1098–101.
- [79] García-Martínez JM, Alessi DR. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem J*. 2008 Dec 15;416(3):375–85.
- [80] Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, et al. Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J*. 1996 Dec 2;15(23):6541–51.

- [81] Martin KA, Rzucidlo EM, Merenick BL, Fingar DC, Brown DJ, Wagner RJ, et al. The mTOR/p70 S6K1 pathway regulates vascular smooth muscle cell differentiation. *Am J Physiol Cell Physiol*. 2004 Mar;286(3):C507–17.
- [82] Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, et al. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev*. 2004 Dec 1;18(23):2893–904.
- [83] Humar R, Kiefer FN, Berns H, Resink TJ, Battegay EJ. Hypoxia enhances vascular cell proliferation and angiogenesis in vitro via rapamycin (mTOR)-dependent signaling. *FASEB J*. 2002 Jun;16(8):771–80.
- [84] Goncharov DA, Kudryashova TV, Ziai H, Ihida-Stansbury K, DeLisser H, Krymskaya VP, et al. Mammalian target of rapamycin complex 2 (mTORC2) coordinates pulmonary artery smooth muscle cell metabolism, proliferation, and survival in pulmonary arterial hypertension. *Circulation*. 2014 Feb 25;129(8):864–74.
- [85] Krymskaya VP, Snow J, Cesarone G, Khavin I, Goncharov DA, Lim PN, et al. mTOR is required for pulmonary arterial vascular smooth muscle cell proliferation under chronic hypoxia. *FASEB J*. 2011 Jun;25(6):1922–33.
- [86] Goncharova EA. mTOR and vascular remodeling in lung diseases: current challenges and therapeutic prospects. *FASEB J*. 2013 May;27(5):1796–807.
- [87] Dai Z, Li M, Wharton J, Zhu MM, Zhao Y-Y. Prolyl-4 hydroxylase 2 (PHD2) deficiency in endothelial cells and hematopoietic cells induces obliterative vascular remodeling and severe pulmonary arterial hypertension in mice and humans through hypoxia-inducible factor-2 α . *Circulation*. 2016 Jun 14;133(24):2447–58.
- [88] Houssaini A, Abid S, Derumeaux G, Wan F, Parpaleix A, Rideau D, et al. Selective tuberous sclerosis complex 1 gene deletion in smooth muscle activates mammalian target of rapamycin signaling and induces pulmonary hypertension. *Am J Respir Cell Mol Biol*. 2016 Sep;55(3):352–67.
- [89] Wessler JD, Steingart RM, Schwartz GK, Harvey BG, Schaffer W. Dramatic improvement in pulmonary hypertension with rapamycin. *Chest*. 2010 Oct;138(4):991–3.
- [90] Seyfarth H-J, Hammerschmidt S, Halank M, Neuhaus P, Wirtz HR. Everolimus in patients with severe pulmonary hypertension: a safety and efficacy pilot trial. *Pulm Circ*. 2013 Sep;3(3):632–8.

